

- B- Cont'd. of Ex. 1
- a) randomly mutating a nucleic acid molecule encoding [wild-type] type 14 pneumolysin polypeptide to produce a mutated nucleic acid molecule[s] encoding modified pneumolysin polypeptide[s] and expressing the mutated nucleic acid molecule[s] in a host cell[s];
 - b) assaying the modified polypeptide expressed by the host cell[s] for hemolytic activity; and
 - c) identifying a [the] modified refoldable pneumolysin polypeptide[s] having [substantially] similar molecular weight as native [wild-type] type 14 pneumolysin [and which are refoldable].

B- Cont'd. of Ex. 1

2. (Amended) A modified [properly refolded] refoldable pneumolysin polypeptide having attenuated hemolytic activity comprising an amino acid sequence of type 14 pneumolysin wherein at least one amino acid in the region comprising amino acids 1 to 257 (SEQ ID NO:3), and besides a substitution of threonine for isoleucine at amino acid 172, is substituted and wherein at least one of said amino acid substitution results in attenuation of the hemolytic activity of the modified pneumolysin polypeptide.

Claim 4, line 3 after "Formula I" insert -- SEQ ID NO: 3 --.

Claim 4, at the end of the claim delete "SEQ ID NO: 3."

Claim 4, line 6 after "and 257", insert -- , said Formula I comprising --.

Claim 5, line 5 after the recitation of the term "lysine", insert -- , --.

Claim 6, line 4 delete the term "cystine", and substitute therefor the term -- cysteine --.

Claim 6, line 5 after the recitation of the term "lysine", insert -- , --.

Claim 13, at the end of the claim insert a period.

Claim 22, line 1 delete "claim 1", and substitute therefor -- claim 2--.

23. (Amended) The modified pneumolysin conjugate of claim 22, wherein the polysaccharide is derived from a [bacteria] bacterium selected from the group consisting of [a] [Haemophilus influenzae] Haemophilus influenzae type b; [meningococcal] meningococcus group A, B or C; group B streptococcus types Ia, Ib, II, III, V or VIII; and [pneumococcal] pneumococcus.

24. (Amended) A vaccine comprising [at least one] a pneumolysin polypeptide of claim [1] 2 and a pharmaceutically acceptable carrier.

26. (Amended) The vaccine according to claim 25, wherein the polysaccharide is a bacterial polysaccharide and is derived from a [bacteria] bacterium selected from the group consisting of [Haemophilus influenzae] Haemophilus influenzae type b; meningococcus group A, B, or C; group A streptococcus or group B streptococcus [serotypes] types Ia, Ib, II, III, V, or VIII; [or] and one or more of serotypes 1-23 of *S. pneumoniae*.

Please add the following claims

-- 31. The modified pneumolysin polypeptide according to claim 2, wherein the hemolytic activity is less than about 1% of the hemolytic activity exhibited by wild-type pneumolysin.

32. The modified pneumolysin polypeptide according to claim 3, wherein the hemolytic activity is less than about 1% of the hemolytic activity exhibited by wild-type pneumolysin.

B.S. Concl.

33. The modified pneumolysin polypeptide having attenuated hemolytic activity according to claim 2 comprising an amino acid sequence of type 14 pneumolysin wherein the region comprising amino acid residues 1 to 257 of SEQ ID NO: 3 is substituted at one or more residue(s) selected from the group consisting of positions 61; 148; and 195; or the combination of positions 17, 18, 61, 66 and 101; 41, 172, 195 and 225; 63, 127, 128 and 148; 33, 46, 83, 239 and 257; and 45, 102, 189 and 195 and wherein at least one of said amino acid substitutions results in attenuation of the hemolytic activity of the modified pneumolysin polypeptide.

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200, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000*

34. The modified pneumolysin polypeptide according to claim 2, wherein the polypeptide is selected from the group consisting of pNVJ1, pNVJ20, pNVJ22, pNVJ45, pNVJ56, pNV103, pNV207, pNV111, and pNV211. --

REMARKS

Claims 1-30 are pending. Claims 12, 14 and 15 stand allowed. Claim 13 stands objected to. Claims 1-7 and 22-26 stand rejected. Claims 8-11, 16-21 and 27-30 have been withdrawn from consideration.

Support for additional claims 31 and 32 is found in the specification at, inter alia, page 53, Table 4 which describes polypeptides with hemolytic activity of less than about